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by I. P. Petrash and L. V. Metlitskiy

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VARIATIONS IN RESPIRATION PROCESS DURING GAMMA RADIATION OF PLANT TISSUE

I. P. Petrash, L. V. Metlitskiy
(Presented by Academician A. I. Oparinyy, December 25, 1965).

Respiration in tomato plants of various degrees of ripeness is analyzed during ionizing radiation doses of 300 krad.

According to numerous data in the literature, respiration in plants is not suppressed even for fairly large doses of ionizing radiation. Respiratory gas exchange becomes somewhat stronger right after irradiation, but it soon returns to the norm. The activity of oxidizing enzymes also changes very little in this process. However, the activity of a number of processes connected with the expenditure of energy does become suppressed simultaneously: the synthesis of pigments (Refs. 1, 2) and the formation of periderm (Ref. 3) are slowed down; resistance to infection, which is inherent in a plant tissue, is lowered (Ref. 3). /711*

Since respiration is the basic source of energy for these processes, it is necessary to conduct a more detailed analysis.

The fruits of the tomato plant of various degrees of ripeness, subjected to γ-irradiation of the dose of 300 krad served as the object of our investigations. This is often used in experimental work on the radio-pasteurization of fruits.

Also in our experiments the intensity of respiration was somewhat enhanced only during the first few days after the irradiation. Neither did we discover any suppression of the activity of a number of oxidizing enzymes: cytochromoxidase, ascorbinoxidase, peroxidase, and polyphenoloxidase (Table 1). On the contrary, we noticed a significant activization of peroxidase, especially in green and whitish fruits. Polyphenoloxidase was slightly active, but even it was not inactivated by the irradiation.

The data on the intensity of respiration present little information on its qualitative aspect, and the activity of enzymes, determined in vitro (as was done in the overwhelming majority of papers), poorly reflects their activity in an intact cell (Ref. 4). In the first place, this refers to the terminal oxidases of the electron-transporting chain, since their normal activity is possible only when they have a specific, mutual orientation (Refs. 5, 6).

Therefore, we have conducted a series of experiments to investigate the qualitative changes in intact cells in the respiratory chain, arising

* Numbers in the margin indicate pagination in the original foreign text.

TABLE 1

CHANGES IN THE ACTIVITY OF TOMATO OXIDIZING ENZYMES UNDER THE INFLUENCE OF GAMMA RAYS

Degree of ripeness of the fruit -- their coloration	Dose of irradiation, in krad	Activity of cytochromoxidase, arbitrary unit	Activity of peroxidase, arbitrary unit	Activity of ascorbinoxidase, in μℓ of O_2/g·hour
Green	0 (control fruit)	0.0063	2.73	124.5
	300	0.0068	3.96	145.3
Whitish	0 (control fruit)	0.0065	3.74	101.7
	300	0.0070	4.36	108.4
Rose	0 (control fruit)	0.0036	2.68	61.4
	300	0.0047	2.93	68.2

during irradiation. For this purpose, we have utilized the specific inhibitors of separate groups of respiratory enzymes: cyanide, azide, antimycin a, whose points of operation are well known (Ref. 7). We have chosen the inhibitors of the terminal oxidases, since it is precisely this segment of the electron-transporting chain which is most productive in terms of energy (Ref. 6).

The analysis was performed on slices of wall tissues from the seed cavities of tomatoes in a Warburg apparatus. The incubation conditions were: 40 min, 30°, phosphate buffer pH 6.6 (using cyanide and azide) and 6.8 (using antimycin a). We have taken the difference which did not exceed 10% of the average value as an admissible divergence between parallel determinations. /712

The experiments were conducted according to two schemes. In the first series of experiments, we subjected green fruit to irradiation, and then -- based on the degree of their ripening -- we kept track of the qualitative changes in the respiration. In the second series, we irradiated tomatoes of various degrees of ripeness, and determined changes in them in the relative role of various respiratory enzymes. The results of the two series of experiments agree well with each other (Table 2). Under the influence of ionizing radiation the role of the enzymes, containing heavy metals in the prosthetic group and suppressed with cyanide, sharply decreases. All the enzymes that have been studied by us are sensitive to cyanide, and, as was already noted (Table 1), they do not lose their activity during irradiation. At the same time, their participation in the absorption of oxygen drops from 80% in the control fruit to 0-10% in the irradiated fruit (Table 2). Ionizing radiation does not directly damage the respiratory enzymes, but they disrupt the normal pattern of their action. The disruption in the respiratory chain is more pronounced, the less ripe are the tomatoes that are subjected to irradiation. This is correlated with greater

TABLE 2

THE EFFECT OF CYANIDE ($5 \cdot 10^{-3}$ M) ON THE ABSORPTION OF OXYGEN BY TOMATO TISSUES

Degree of ripeness of the fruit at the time of irradiation - their coloration	Irradiation dose, in krad	Residual respiration, according to stages of ripening, %			
		Green	Whitish	Rose	Red
Green	0 (control fruit)	21.9	33.2	58.2	71.4
	300	90.7	112.4	85.9	96.3
Whitish	0 (control fruit)	-	44.0	63.7	74.1
	300	-	99.3	91.5	92.0
Rose	0 (control fruit)	-	-	63.3	78.5
	300	-	-	88.6	95.2
Red	0 (control fruit)	-	-	-	69.2
	300	-	-	-	95.0

disruptions in the rate of ripening and the degree of resistance to phytopathogenic microorganisms in green fruit, as compared with ripe ones (Refs. 2, 1, 6, 8).

The application of other respiration inhibitors confirmed the data obtained when cyanide was utilized (Table 3).

TABLE 3

EFFECT OF AZIDE AND ANTIMYCIN A ON THE ABSORPTION OF O_2 BY TOMATO TISSUES

Inhibitor	Dose of irradiation, krad	Residual respiration, according to stages of ripening, %		
		Green	Whitish	Rose
Azide ($5 \cdot 10^{-3}$ M)	0 (control fruit)	36.4	32.2	42.6
	300	84.2	70.5	65.6
Antimycin a (8 μ gram)	0 (control fruit)	49.5	63.9	81.0
	300	104.7	124.8	115.4

After the irradiation, we observed a sharp decrease in the sensitivity of respiration to azide, which suppresses the metal-containing enzymes, and to antimycin a, which interrupts the transport of electrons between the cytochromes b_1 and c_1.

Thus, ionizing radiation strongly influences the terminal oxidases of the respiratory chain -- in particular, cytochromes. Since the intensity of respiration is not lowered in this process, there is a basis to assume that during irradiation and other types of influence (change of temperature, mechanical injuries, infection, etc.), the principal way that electrons are transported through cytochromes -- cytochrome oxidase -- is replaced by alternative ways which are less effective in terms of energy.

In the case of non-radiation influences, it is assumed that the transfer of electrons to oxygen may be accomplished either by flavoprotein oxidases or by autooxidabelian cytochromes from b group (Refs. 9, 10). Possibly, the cyanide-resistant respiration of irradiated tissues is also accomplished by these enzymes. A significant activization of peroxidase in the irradiated fruit /713 may be considered as an indirect confirmation of this assumption (Table 1), since usually the activities of the flavoprotein oxidases and those of peroxidase are interlinked.

In the literature, data have been appearing repeatedly on the radiosensitivity of cytochromes (Ref. 10), and on the possible role of the change in respiration to alternative ways when the cell is injured by radiation (Ref. 11). It also seems probable, however, that a change in the terminal oxidases of the electron-transporting chain is important not only for the development of radiation injury, but also for the healing processes. We have the following basis for this assumption.

First of all, a change of the enzymatic systems in the respiratory chain is observed in the course of plant growth, and as a response reaction to injuries. Consequently, at a specific stage of the radiation injury the change in oxidation to alternative ways may represent a physiological change, guaranteeing the healing of the injuries.

Secondly, recently it has been shown that yeast, which is deficient in cytochromes, is incapable of post-radiation regeneration (Ref. 12). This may be partly true because it does not have any auxiliary way of transporting electrons. Finally, we have observed that the accumulation of carotenoids continued in the fruit after its irradiation with a dose of 300 krad (although more slowly than in the control fruit). The normal reaction to mechanical injuries, and some ability to resist infection were also preserved. Consequently, an auxiliary way of transporting electrons can quarantee the metabolism in the irradiated fruit, although at a lower level as compared with the norm.

The view expressed above requires rigorous experimental confirmation, which is the goal of further research.

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